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Synthesis and Cytotoxicity of 9-Alkoxy-1,5-dichloroanthracene Derivatives in Murine and Human Cultured Tumor Cells

9-Alkoxy-1,5-dichloroanthracenes were sucessfully prepared. Their cytotoxicity was evaluated *in vitro* on rat glioma C6 cell lines and human hepatoma G2 cell lines, respectively. Alkylation of 1,5-dichloro-9(10*H*)-anthracenone with either the appropriate alcohols or alkyl chlorides in the presence of sulfuric acid or sodium hydride, respectively, furnished this structural class of anthracenes. Contrary to mitoxantrone, cytotoxic properties were observed as documented by the reactivity of the novel compounds and potent *in vitro* activity against C6 cells and hep G2 cells over a wide range of structural variants. Among these compounds, **5c**, **5h**, **5l** and **5n** are potent cytotoxins. They inhibit C6 cell growth in culture, indicated by using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide sodium salt (XTT) colorimetric assay. By using this assay it was also shown that **5c**, **5d** and **5l** possess potent cytotoxicity on hep G2 cells. The most active compound displaying *in vitro* cytotoxicity was the 9-butoxy derivative **5h** with IC_{50} values 0.02 μM against C6 cells, as compared with mitoxantrone with IC_{50} values 0.07 μM. The most active compound displaying *in vitro* cytotoxicity against hep G2 cells was **5c** with IC_{50} values 1.7 μM (mitoxantrone: 0.8 μM). Structure-activity relationships (SAR) of these compounds with respect to the nature of the alkoxy substitution in the 9 position are discussed for both cell lines.

Keywords: Anthracene; Cytotoxicity; Rat glioma C6 cells; Human hepatoma G2 cells; Mitoxantrone; XTT colorimetric assay

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Introduction

Anthracene derivatives have been the subject of extensive research mainly due to their well-recognized biological importance and the significant biological applications of their derivatives [1]. Mitoxantrone, an anthracene-9,10-dione, has gained an important position in the clinical management of leukemia and lymphomas as well as in combination therapy of advanced breast and ovarian cancers [2]. We have previously shown that 9-acyloxy-1,5-dichloroanthracenes (**1**), 9-acyloxy-1,8-dichloroanthracenes (**2**) and 10-substituted 1,5-dichloro-9(10*H*)-anthracenones (**3**, **4**) provide useful templates for the design of potent antitumor derivatives [3, 4] (Scheme 1). We have recently reported the cytotoxicity, lipid peroxidation activity and telomerase inhibition of some of these analogs in murine and human tumor cultured cells, including suspended as well as selected solid tumors. Structure-activity relationships (SAR) including the position of substituents, chemical and physical properties, lipophilicity-hydrophilicity balance, etc., still need to be investigated. Our SAR study was mainly focused on the modification of the substituent at the anthracene system at the C-9, C-10 and other positions. Despite the extensive and long-standing therapeutic utilization of anthracenones and anthracenes, their mechanism of action is still uncertain. Many cell types [5–7] and cellular macromolecules [8–12] have been identified as potential targets of anthracenones, and a number of different mechanisms have been proposed for the antiproliferative and anti-inflammatory effects of these agents. These include interaction with DNA, inhibition of various enzyme systems associated with cell proliferation and inflammation, and alteration of mitochondrial functions and destruction of membrane lipids [8]. Part of the difficulty may lie in the historical preference for carrying out cytotoxic screening regimens *in vivo* or *in vitro*. Many useful therapeutic drugs have their discoveries rooted in serendipity. An encouraging trend has been to elucidate the molecular origin of the proinflammatory action of the anthrone class and, by appropriate chemical modification, to improve the therapeutic index [13]. There also have been several studies to evaluate anthracenes, anthracenones and anthraquinones for potential antitumor properties with much of the work involving interca-

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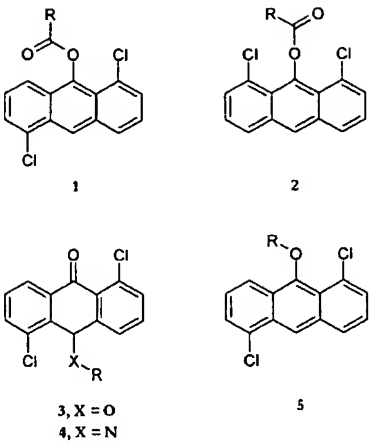
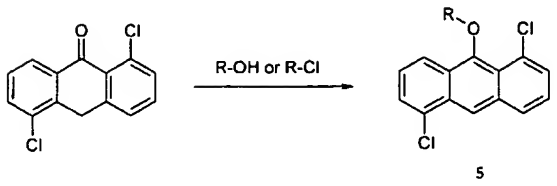


Scheme 1



Method A:

Reagents: R-OH, benzene, H_2SO_4;

R = CH_2CH_2Br; $(CH_2)_nCH_3$, n = 1,2,3,7; $(CH_2)_nCH_2Cl$, n = 1,2,3; $(CH_2)_nCH(CH_3)_2$, n = 1,2; $CH_2CH_2C_6H_5$.

Method B:

Reagents: R-Cl, NaH, THF;

R = CH_3, $CH_2C_6H_5$, $CH_2C_6H_4CH_3(o)$, $CH_2C_6H_4CH_3(p)$.

Scheme 2

lating agents [14–19]. Nevertheless, with an alkoxy moiety attached to the anthracene pharmacophore at C-9, it is believed that compounds having biological activity could be obtained. Our research program in this area, like others, has been hampered by the lack of synthetic methods for the preparation of substituted anthracene compounds. Thus, the search for new chromophore-modified molecules with anti-proliferative properties is a very active domain of research. Here two different simple methodologies are reported for the synthesis of 9-alkoxy-1,5-dichloroanthracene derivatives, thereby providing some additional insight into the bioactive nature of the substituted anthracene molecules.

Results

The 9-alkoxy-1,5-dichloroanthracene analogues **5a–o** were prepared by reaction of 1,5-dichloro-9(10*H*)-anthracenone with appropriate alcohols in the presence of sulfuric acid (Scheme 2, method A, acid catalysis) or alkyl chlorides in the presence of NaH (Scheme 2, method B, base catalysis). In both cases alkylation takes place at C-9. The Williamson synthesis [17] involves nucleophilic substitution of alkoxide ion or phenoxide ion for a halide ion. This synthesis can be used in this process to make unsymmetrical ether derivatives. Scheme 2 illustrates the synthetic methods for these target compounds. Elucidation of the mechanism of how acid catalysis or base catalysis achieves this desirable selectivity has gained considerable interest. Barnett et al. [18] investigated the *p*-toluenesulfonic acid catalyzed formation and dehydration of anthrone hemiacetals, found such reactions to occur incompletely, and went on to develop transetherification of 9-methoxyanthracene. The one-step dehydration procedure in method A becomes feasible for the preparation of primary 9-alkoxy-1,5-dichloroanthracenes when a large excess of a primary alcohol is used with sulfuric acid catalysis in benzene and the water byproduct is removed by azeotropic distillation [19]. In an alternative method (method B), alkyl chlorides and NaH were used for alkylation of the 9-position in refluxing THF. Base catalysis can be explained by assuming rapid attack of R-Cl on anthracenone after deprotonation by NaH [20]. Interestingly, regioselective alkylation of anthracenone was achieved by acid catalysis, except **5a**, **5l**, **5m** and **5n** which were obtained under base catalysis. Details on various reaction conditions and solvents used are described in the experimental section. The proof of structures was based on IR, MS, ^{1}H NMR, ^{13}C NMR and elemental analysis. The ^{13}C NMR spectra of these compounds lack the carbonyl resonance near δ 183.7 [21, 22], but show absorption in the δ 150.40–153.09 region for C-9. In addition, ^{1}H NMR and ^{13}C NMR correlation of these and other spectra allowed structure assignments (experimental section).

Discussion

The primary goal of the present study was to compare the cytotoxic effects of the prepared compounds on rat glioma C6 cells and human hepatoma G2 cells. We determined the cytotoxicity by XTT (a tetrazolium salts) colorimetric assay. 1,5-Dichloroanthracenes bearing 2-chloroethoxy-, butoxy- and *p*-methylbenzyloxy substituents at C-9 have higher antitumor efficacy in these series of compounds against rat glioma C6 cells. The IC_{50} value for the butoxy-substituted compound **5h** showed that it is was actually more potent than the clinical drug, mitoxantrone. The 2-chloroethoxy-, 2-bromoethoxy- and benzyloxy-substituted derivatives show highest cytotoxic activity against human hepatoma G2 cells. The substitu-

Table 1. Cytotoxic activity of 9-alkoxy 1,5-dichloroanthracenes on C6 cells and Hep G2 cells.

Entry	R	IC_{50} [μM][a] C6 cells[b]	Hep G2[c]
5a	CH_3	47.2 ± 1.5	48.1 ± 1.8
5b	CH_2CH_3	15.4 ± 1.1	45.3 ± 1.3
5c	CH_2CH_2Cl	0.9 ± 0.1	1.7 ± 0.2
5d	CH_2CH_2Br	22.3 ± 1.2	18.5 ± 1.1
5e	$CH_2CH_2CH_3$	26.8 ± 1.5	38.6 ± 1.8
5f	$CH_2CH(CH_3)_2$	17.9 ± 1.4	45.6 ± 1.2
5g	$CH_2CH_2CH_2Cl$	11.3 ± 0.8	49.5 ± 1.3
5h	$CH_2CH_2CH_2CH_3$	0.02 ± 0.01	40.6 ± 1.7
5i	$CH_2CH_2CH(CH_3)_2$	19.6 ± 1.6	50.5 ± 1.3
5j	$CH_2CH_2CH_2CH_2Cl$	28.4 ± 1.3	>50
5k	$(CH_2)_7CH_3$	26.7 ± 1.5	36.2 ± 1.8
5l	$CH_2C_6H_5$	5.4 ± 0.3	13.1 ± 1.2
5m	$CH_2C_6H_4CH_3$(o)	29.9 ± 1.5	26.7 ± 1.4
5n	$CH_2C_6H_4CH_3$(p)	0.09 ± 0.01	>50
5o	$CH_2CH_2C_6H_5$	10.7 ± 0.5	43.7 ± 1.6
Mitoxantrone		0.7 ± 0.01	0.8 ± 0.1

[a] IC_{50}, drug concentration inhibiting 50% of cellular growth following 48 h of drug exposure. Values are in μM and represent an average of three experiments. The variance for the IC_{50} was less than ±20%. [b] C6 cells: rat glioma C6 cells. [c] Hep G2: human hepatoma G2 cells.

ents on the anthracene system may affect drug binding in terms of their sequence-specific binding sites and binding constant. The selectivity of cytotoxicity may depend on individual structural components which affect the transport into the tumor cells as well as pharmacodynamics of the agent. *In vitro* testing on rat glioma C6 cell lines and human hepatoma G2 cell lines assay revealed that compounds, O-linked by short alkyl chains or a *p*-methylbenzyloxy group (e.g. **5b**, **5c**, **5d**, **5g**, **5h**, **5l**, **5n** and **5o**), exhibited cytotoxic potency comparable with mitoxantrone and possessed the best effect in dose- and time-dependent characters. It was shown that **5c**, **5d** and **5l** possessed potential anticancer effect on human hepatoma G2 cells by using the XTT colorimetric assay (Table 1). Compounds **5c**, **5h**, **5l** and **5n** showed cytotoxicity on rat glioma C6 cells. Compound **5h** was superior to the lead compound, mitoxantrone. Table 1 shows that the cytotoxicity of **5a**, **5b**, **5d**, **5e**, **5f**, **5g**, **5i**, **5j**, **5m** and **5o** is markedly reduced or not significantly increased with respect to both cell lines. Results from Table 1 show that the 2-chloroethoxy substituted analogue **5c** retains the cytotoxicity against both C6 cells and hep G2 cells. In analogues **5c**, **5g** and **5j** the cytotoxicity is decreased according to the length of the side chain. More elaborate experiments against different cell lines will be carried out. Actually, some compounds are shown to have promising antitumor effects in this testing system. In conclusion: in this series of anthracene derivatives with one substituent at C-9 of the nearly planar tricyclic ring system, compound **5h** with a butoxy group was 3.5 times more cytotoxic than mitoxantrone in C6 cell lines, while **5n** with a *p*-methylbenzyl substituent was slightly less potent than mitoxantrone.

Acknowledgements

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Experimental Section

Materials and Apparatus

Melting points were determined with a Büchi B-545 melting point apparatus and are uncorrected. All reactions were monitored by TLC (silica gel 60 F_{254}), flash column chromatography: silica gel (70–230 mesh, E. Merck, Darmstadt, Germany) with CH_2Cl_2 as eluent. ^1H NMR: Varian GEMINI-300 (300 MHz); δ values are in ppm relative to TMS as an internal

standard. Fourier-transform IR spectra (KBr): Perkin-Elmer 983G spectrometer. Mass spectra (EI, 70 eV, unless otherwise stated): Finnigan MAT TSQ-46 and Finnigan MAT TSQ-700. Elemental analyses (C, H, and N): Universität Münster (Germany), variation of values within 0.4 ppm. Typical experiments illustrating the general procedures for the preparation of the anthracenes are described below.

Synthesis

General procedure for the preparation of 9-alkoxy-1,5-dichloroanthracenes

Method A: To a solution of 1,5-dichloro-9(10*H*)-anthracenone (2.0 mmol) and H_2SO_4 (0.1 mL) in dry benzene (20 mL) was added dropwise a solution of an appropriate alcohol (3 mmol) in dry benzene (10 mL) under N_2 blanketing. The reaction mixture was refluxed for 48 h. Water (250 mL) was added and then the mixture was extracted with dichloromethane. The combined organic extracts were washed with water, dried ($MgSO_4$), and concentrated. The resulting precipitate was collected by filtration, washed with water and purified by chromatography and crystallization.

Method B: To a solution of 1,5-dichloro-9(10*H*)-anthracenone (2.0 mmol) and NaH (3.0 mmol) in dry THF (20 mL) was added dropwise a solution of an appropriate alkyl chloride (3.0 mmol) in dry THF (10 mL) under N_2. The reaction mixture was refluxed for 24 h. Then follow Method A.

9-Methoxy-1,5-dichloroanthracene **5a**

This material was prepared in 25% yield analogously to method B; mp 100–101 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 4.13 (s, 3H), 7.24–7.41 (m, 2H), 7.52–7.60 (m, 2H), 7.94 (dd, *J* = 1.0, 8.5 Hz, 1H), 8.28 (dd, *J* = 1.0, 8.8 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 29.78, 64.58, 120.59, 121.93, 122.32, 125.37, 126.39, 127.37, 128.65, 128.88, 129.19, 129.93, 132.00, 134.56, 153.09; UV λ_{max} (hexane) nm (log ε): 403 (3.78), 381 (3.81), 362 (3.43), 391 (2.28), 370 (2.90); IR (KBr) cm^{-1}: 1350; MS *m/z* 276 (M^+), 261, 233; Anal. Calcd. for $C_{15}H_{10}OCl_2$: C, 65.01, H, 3.63. Found: C, 65.28, H, 3.41.

9-Ethoxy-1,5-dichloroanthracene **5b**

This material was prepared in 46% yield analogously to method A; mp 99–100 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 1.62 (t, *J* = 7.0 Hz, 3H), 4.12 (q, *J* = 7.0 Hz, 2H), 7.24–7.41 (m, 2H), 7.52–7.60 (m, 2H), 7.93 (d, *J* = 8.5 Hz, 1H), 8.26 (d, *J* = 8.9 Hz, 1H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 15.54, 73.24, 120.43, 122.19, 122.43, 125.25, 126.40, 127.62, 128.91, 129.11, 129.89, 131.96, 134.60, 152.02; UV λ_{max} (hexane) nm (log ε): 404 (3.84), 382 (3.88), 363 (3.56), 392 (2.89), 371 (3.24); IR (KBr) cm^{-1}: 1335; MS *m/z* 291 (M^+), 262, 227, 198, 163; Anal. Calcd. for $C_{16}H_{12}OCl_2$: C, 65.01, H, 3.63. Found: C, 65.28, H 3.41.

9-(2-Chloroethoxy)-1,5-dichloroanthracene **5c**

This material was prepared in 69% yield analogously to method A; mp 149–150 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 4.04 (t, *J* = 5.6 Hz, 2H), 4.33 (t, *J* = 5.6 Hz, 2H), 7.34 (dd, *J* = 7.5, 8.0 Hz, 1H), 7.43 (dd, *J* = 7.2, 8.6 Hz, 1H,), 7.56 (d, *J* = 7.1 Hz, 1H), 7.61 (d, *J* = 7.1 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 8.45 (d, *J* = 8.8 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 42.87, 76.26, 119.72, 121.26, 122.19, 125.37, 125.79, 126.57, 127.41, 129.07, 129.45, 129.85, 131.12, 131.98, 134.76, 150.54; UV λ_{max} (hexane) nm (log ε): 402 (3.69), 381 (3.73), 361 (3.48), 345 (3.09), 370 (3.24); IR (KBr) cm^{-1}: 1330; MS *m/z* 325 (M^+), 261; Anal. Calcd. for $C_{16}H_{11}OCl_3$: C, 59.01, H, 3.40. Found: C, 59.27, H, 3.52.

9-(2-Bromoethoxy)-1,5-dichloroanthracene **5d**

This material was prepared in 69% yield analogously to method A; mp 126–127 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 3.87 (t, *J* = 6.0 Hz, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 7.24–7.43 (m, 2H), 7.51–7.60 (m, 2H), 7.92 (d, *J* = 8.1 Hz, 1H), 8.42 (d, *J* = 9.0 Hz, 1H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 30.04, 76.46, 121.19, 121.94, 122.12, 125.30, 125.70, 126.51, 127.32, 128.23, 129.00, 129.40, 129.73, 131.93, 134.38, 150.40; UV λ_{max} (hexane) nm (log ε): 403 (3.74), 381 (3.78), 361 (3.56), 391 (3.21), 370 (3.36); IR (KBr) cm^{-1}: 1335; MS *m/z* 370 (M^+), 261, 233, 163; Anal. Calcd. for $C_{16}H_{11}OBrCl_2$: C, 51.89, H, 2.97. Found: C, 51.57, H, 3.15.

9-Propoxy-1,5-dichloroanthracene **5e**

This material was prepared in 42% yield analogously to method A; mp 54–55 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 1.16 (t, *J* = 6.5 Hz, 3H), 1.99–2.17 (m, 2H), 4.00 (t, *J* = 6.8 Hz, 2H), 7.24–7.39 (m, 2H), 7.50–7.58 (m, 2H), 7.91 (d, *J* = 8.4 Hz, 1H), 8.26 (d, *J* = 8.8 Hz, 1H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.66, 23.44, 79.17, 120.35, 122.19, 122.41, 125.18, 125.21, 126.34, 127.52, 128.82, 128.89, 129.03, 129.89, 131.95, 134.57, 152.13; UV λ_{max} (hexane) nm (log ε): 404 (3.77), 382 (3.81), 363 (3.51), 392 (2.89), 371 (3.19); IR (KBr) cm^{-1}: 1332; MS *m/z* 305 (M^+), 262, 227, 163; Anal. Calcd. for $C_{17}H_{14}OCl_2$: C, 66.90, H, 4.62. Found: C, 67.18, H, 4.77.

9-Isopropoxy-1,5-dichloroanthracene **5f**

This material was prepared in 41% yield analogously to method A; mp 55–56 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 1.19 (d, *J* = 6.6 Hz, 6H), 2.49 (m, 1H), 3.82 (d, *J* = 6.9 Hz, 2H), 7.28–7.42 (m, 2H), 7.52–7.61 (m, 2H), 7.95 (d, *J* = 8.3 Hz, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR: ($CDCl_3$) δ: 19.65, 29.31, 83.96, 120.43, 122.30, 122.45, 125.21, 125.27, 126.38, 127.52, 128.94, 129.05, 129.98, 131.98, 134.64, 152.29; UV λ_{max} (hexane) nm (log ε): 404 (3.86), 383 (3.90), 363 (3.69), 392 (3.35), 372 (3.50); IR (KBr) cm^{-1}: 1334; MS *m/z* 319 (M^+), 262, 227, 163; Anal. Calcd. for $C_{18}H_{16}OCl_2$: C 67.72, H 5.05. Found: C 67.58, H 4.91.

9-(3-Chloropropoxy)-1,5-dichloroanthracene **5g**

This material was prepared in 75% yield analogously to method A; mp 101–102 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 2.42–2.54 (m, 2H), 3.98 (t, *J* = 6.4 Hz, 2H), 4.16 (t, *J* = 6.0 Hz, 2H), 7.24–7.40 (m, 2H), 7.49–7.58 (m, 2H), 7.89 (d, *J* = 8.2 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 33.14, 41.78, 73.52, 120.79, 122.10, 125.23, 125.47, 126.41, 127.34, 128.49, 128.96, 129.22, 129.82, 131.98, 134.48, 151.36; UV λ_{max} (hexane) nm (log ε): 403 (3.99), 382 (4.03), 362 (3.84), 391 (3.52), 371 (3.66); IR (KBr) cm^{-1}: 1330; MS *m/z* 339 (M^+), 262, 227, 163; Anal. Calcd. for $C_{17}H_{13}OCl_3$: C, 60.17, H, 3.83. Found: C, 59.97, H, 3.65.

9-Butoxy-1,5-dichloroanthracene **5h**

This material was prepared in 45% yield analogously to method A; mp 79–80 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 1.04 (t, *J* = 7.3 Hz, 3H), 1.54–1.73 (m, 2H), 2.01–2.12 (m, 2H), 4.05 (t, *J* = 6.8 Hz, 2H), 7.24–7.41 (m, 2H), 7.56 (t, *J* = 7.9 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.21, 19.45, 32.33, 76.46, 120.37, 122.21, 122.43, 125.20, 125.25, 126.36, 127.56, 128.91, 129.05, 129.93, 131.96, 134.62, 152.22; UV λ_{max} (hexane) nm (log ε): 404 (3.75), 382 (3.83), 363 (3.49), 392 (2.90), 371 (3.20); IR (KBr) cm^{-1}: 1330; MS *m/z* 319 (M^+), 262, 227, 163; Anal. Calcd. for $C_{18}H_{16}OCl_2$: C, 67.72, H, 5.05. Found: C, 67.96, H, 5.11.

9-Isobutoxy-1,5-dichloroanthracene **5i**

This material was prepared in 38% yield analogously to method A; mp 73–74 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 0.98–1.05 (m, 6H), 1.19–1.22 (m, 1H), 1.95–2.01 (m, 2H), 4.07 (t, *J* = 7.0 Hz), 7.27–7.42 (m, 2H), 7.52–7.60 (m, 2H), 7.94 (d, *J* = 8.5 Hz, 1H), 8.28 (dd, *J* = 4.0, 10.0 Hz, 1H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 22.96, 25.24, 39.03, 82.85, 120.41, 122.21, 122.41, 122.47, 125.25, 126.40, 127.60, 128.85, 128.94, 129.09, 129.94, 132.00, 134.64, 152.27; UV λ_{max} (hexane) nm (log ε): 404 (3.83), 383 (3.87), 363 (3.61), 392 (3.16), 372 (3.36); IR (KBr) cm^{-1}: 1327; MS *m/z* 333 (M$^+$), 262, 227, 163; Anal. Calcd. for $C_{19}H_{18}OCl_2$: C, 68.47, H, 5.44. Found: C, 68.54, H, 5.47.

9-(4-Chlorobutyl)oxy-1,5-dichloroanthracene **5j**

This material was prepared in 59% yield analogously to method A; mp 73–74 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 2.07–2.24 (m, 4H), 3.69 (t, *J* = 5.9 Hz, 2H), 4.03 (t, *J* = 5.8 Hz, 2H), 7.27–7.38 (m, 2H), 7.49–7.57 (m, 2H), 7.89 (d, *J* = 8.5 Hz, 1H), 8.17 (d, *J* = 8.9 Hz, 1H), 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 27.69, 29.50, 45.02, 76.46, 120.55, 122.07, 125.23, 125.34, 126.34, 127.34, 128.56, 128.91, 129.13, 129.82, 132.00, 134.48, 151.76; UV λ_{max} (hexane) nm (log ε): 403 (4.01), 382 (4.05), 362 (3.85), 391 (3.54), 371 (3.68); IR (KBr) cm^{-1}: 1331; MS *m/z* 353 (M$^+$), 262, 227, 163, 91, 55.

9-Octyloxy-1,5-dichloroanthracene **5k**

This material was prepared in 68% yield analogously to method A; mp 65–66 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 0.89 (t, *J* = 6.7 Hz, 3H), 1.24–1.43 (m, 8H), 1.55–1.61 (m, 2H), 2.02–2.08 (m, 2H), 4.05 (t, *J* = 6.9 Hz, 2H), 7.31–7.40 (m, 2H), 7.54–7.60 (m, 2H), 7.95 (d, *J* = 8.5 Hz, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.62 (s, 1H); UV λ_{max} (hexane) nm (log ε): 404 (3.99), 383 (4.03), 363 (3.83), 392 (3.52), 372 (3.66); IR (KBr) cm^{-1}: 1330; MS *m/z* 374 (M$^+$), 262; Anal. Calcd. for $C_{22}H_{24}OCl_2$: C, 70.40, H, 6.44. Found: C, 70.12, H, 6.21.

9-Benzyloxy-1,5-dichloroanthracene **5l**

This material was prepared in 32% yield analogously to method B; mp 130–131 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 5.15 (s, 2H), 7.27–7.53 (m, 5H), 7.58–7.69 (m, 4H), 8.00 (d, *J* = 8.2 Hz, 1H), 8.29 (d, *J* = 8.9 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 78.72, 120.85, 122.39, 125.34, 125.43, 126.43, 127.52, 127.80, 128.12, 128.33, 128.65, 128.78, 128.94, 129.23, 129.91, 132.00, 134.55, 137.15, 151.60; UV λ_{max} (hexane) nm (log ε): 404 (3.74), 382 (3.78), 362 (3.51), 392 (3.07), 371 (3.24); IR (KBr) cm^{-1}: 1331; MS *m/z* 353 (M$^+$), 91; Anal. Calcd. for $C_{21}H_{14}OCl_2$: C, 71.40, H, 3.99. Found: C, 71.66, H, 4.17.

9-(2-Methylbenzyloxy)-1,5-dichloroanthracene **5m**

This material was prepared in 35% yield analogously to method B; mp 119–120 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 2.23 (s, 3H), 5.16 (s, 2H), 7.21–7.37 (m, 5H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.86 (d, *J* = 7.4 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 8.16 (d, *J* = 8.9 Hz, 1H), 8.68 (s, 1H); UV λ_{max} (hexane) nm (log ε): 404 (3.73), 382 (3.76), 363 (3.23), 392 (3.00), 371 (3.14); IR (KBr) cm^{-1}: 1335; MS *m/z* 366 (M$^+$), 262, 105; Anal. Calcd. for $C_{22}H_{16}OCl_2$: C, 71.94, H, 4.39. Found: C, 71.71, H, 4.43.

9-(4-Methylbenzyloxy)-1,5-dichloroanthracene **5n**

This material was prepared in 38% yield analogously to method B; mp 134–135 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H), 5.10 (s, 2H), 7.26–7.37 (m, 4H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.58 (t, *J* = 6.1 Hz, 2H), 7.98 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.9 Hz, 1H), 8.67 (s, 1H); UV λ_{max} (hexane) nm (log ε): 404 (3.91), 382 (3.95), 363 (3.74), 392 (3.45), 371 (3.56); IR (KBr) cm^{-1}: 1335; MS *m/z* 366 (M$^+$), 262, 105; Anal. Calcd. for $C_{22}H_{16}OCl_2$: C, 71.94, H, 4.39. Found: C, 71.78, H, 4.18.

9-Phenylethoxy-1,5-dichloroanthracene **5o**

This material was prepared in 55% yield analogously to method A; mp 90–91 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 3.34 (t, *J* = 7.1 Hz, 2H), 4.24 (t, *J* = 7.1 Hz, 2H), 7.18–7.39 (m, 7H), 7.51–7.56 (m, 2H), 7.85–7.94 (m, 2H), 8.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 36.70, 76.46, 120.57, 122.30, 125.25, 126.34, 126.60, 127.45, 128.53, 128.74, 128.93, 129.13, 129.40, 129.85, 131.86, 134.53, 138.32, 151.69; UV λ_{max} (hexane) nm (log ε): 404 (3.91), 382 (3.95), 363 (3.72), 392 (3.36), 371 (3.50); IR (KBr) cm^{-1}: 1335; MS *m/z* 367 (M$^+$), 262, 105; Anal. Calcd. for $C_{22}H_{16}OCl_2$: C, 71.94, H, 4.39. Found: C, 71.78, H, 4.66.

Pharmacological studies

Cell culture

C6 cells (rat glioma cells) and Hep G2 cells (human hepatoma carcinoma) were cultured in minimum essential medium (MEM), supplemented with 10% fetal calf serum, 100 units/mL penicillin and 100 mg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Cell culture media were renewed every three days, up to the confluence of the monolayer. Cell cultures were passaged when they had formed confluent cultures, using trypsin-EDTA to detach the cells from their culture flasks or dishes.

Cytotoxicity measurement: XTT assay [23–26]

The tetrazolium reagent (XTT; 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide sodium salt, Sigma, MI, USA) was designed to yield a suitably colored, water-soluble, non-toxic formazan upon metabolic reduction by viable cells. Approximately 2×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in an humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. Crystalline stock test compounds were stored at –70 °C and dissolved in 1% DMSO. All the drug solutions were prepared immediately before use and were diluted by complete medium. Test drug solutions were then added to the culture medium at various concentrations. After 72 h, fresh XTT (50 µL) and electron coupling reagent (phenazine methosulfate, PMS; Sigma, MI, 1 µL) were mixed together, and 50 µL of this mixture were added to each well. After incubation at 37 °C for 6 h, the absorbency at 490 nm was measured with the ELISA reader.

Statistics

The mean and standard deviation are designated by "X ± SD". The probable level of significance ($p \leq 0.05$) between test and control sample was determined by the Student's "*t*" test with the raw data.

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